ZOOLOGICAL SCIENCE 7: 551–555 (1990)

[COMMUNICATION]

Immunocytochemical and Ultrastructural Characterization of the Cells in the Pars Tuberalis of the Turtle, *Geoclemys reevesii*

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ABSTRACT—Using immunocytochemical and electron microscopical techniques, two distinct types of secretory cells were detected in the turtle pars tuberalis (PT). The cells showing positive immunoreaction to TSH-antiserum were exclusively found in the rostral PT, while those immunoreactive to FSH-antiserum were concentrated in the caudal PT.

INTRODUCTION

The identification of cell types producing different pituitary hormones has been established in the pars distalis (PD) of the reptilian pituitary gland by conventional staining methods [1–3], electron microscopy [2, 4, 5] and immunocytochemistry [5–8]. However, little attention has been paid to the pars tuberalis (PT), since PT is predominantly composed of chromophobic cells. A few ultrastructural studies showed the presence of secretory granules in some cells in the PT of the turtle [6, 8, 9]. Thus, to date, there is very little information concerning the functional significance of the PT. The present report describes the presence of cells immunoreactive to FSH- or TSH-antiserum in the turtle PT.

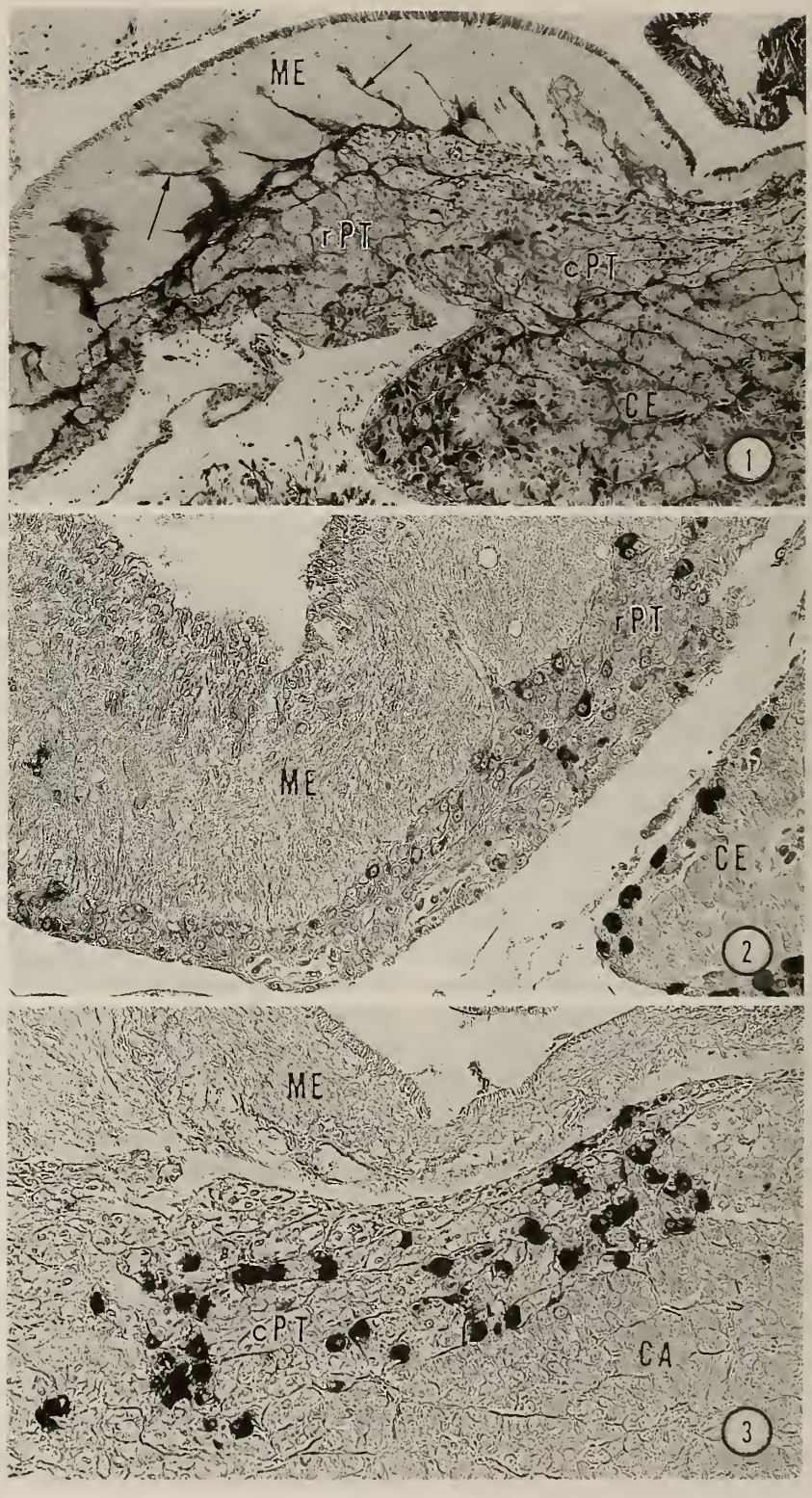
MATERIALS AND METHODS

Adult male and female turtles, *Geoclemys reevesii*, (caraspace length, 160–200 mm) were obtained from a dealer. The hypothalamic regions of 6 animals were fixed in Bouin-Hollande sublimate solution. Sections were made at 6 μm thickness through ordinary paraffin method. Some sections from each animal were stained with Azan or Gomori's paraldehyde fuchsin staining method. For immunocytochemical study, avidin-biotin-peroxidase complex method was used [10]. The following antisera raised against rabbits were used: anti-rat somatotropin, anti-rat prolactin, anti-rat TSH-β, anti-porcine ACTH, anti-bullfrog LH-β and anti-bullfrog FSH-β. All the primary antisera were kindly provided by Dr. K. Wakabayashi, Hormone Assay Center, Institute of Endocrinology, Gunma University. To ensure the specificity, all positive results were controlled by omission of the primary antisera and by a parallel incubation with antisera preabsorbed with the respective hormone. For electron microscopy, the hypothalamic regions of 8 animals were fixed in paraformaldehyde and glutaraldehyde, postfixed in 1% OsO_4, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEM-T8 electron microscope.

RESULTS AND DISCUSSION

In the turtle, the PT is well developed and can be divisible into rostral and caudal portions (Fig. 1). The rostral portion spreads under the median eminence as a thick layer of flattened epithelial cells. Between the rostral portion and the median eminence, lie capillaries of the primary plexus, whose blood drains into the hypophysial portal

Accepted September 8, 1989
Received August 15, 1989


ME
rPT
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vessels. The caudal portion is a thick cluster of cells situated dorsally to the anterior part of the PD. Histologically, the majority of the tuberal cells are chromophobic for the tinctorial stainings used in this study. A few cells showing slight affinity for AF appear only in the caudal portion of the PT, although there are numerous AF-positive cells in the PD.

In the rostral portion, some cells show a positive reaction to TSH-antiserum (Fig. 2). They are large and angular in shape. Cells reacting to FSH-antiserum are found to be concentrated mainly in the caudal portion, generally being spherical or ovoid in shape (Fig. 3). Preabsorption of the antisera with TSH and FSH and also omission of the respective primary antisera completely block the positive staining results. Throughout their distributions, the immunoreactive FSH-cells outnumber the immunoreactive TSH-cells (Figs. 2, 3). All the tuberal cells are non-reactive to other antisera than TSH-and FSH- antisera tested.

Although the PT tissue appears largely chromophobic with tinctorial staining methods it is very interesting to note that two types of cells were identified immunocytochemically: cells showing TSH-like immunoreactivity entirely in the rostral portion and cells showing FSH-like immunoreactivity largely in the caudal portion. In the PD, TSH- and FSH- immunoreactive cells have been frequently demonstrated in the caudal lobe of the turtle PD [5]. Since the turtle PT develops as a pair of lateral outgrowths from the anterior aspects of the pituitary anlage [7], it is possible that the cells reacting with TSH- and FSH- antisera in the PT might have migrated from the caudal lobe of the PD during embryogenesis.

Five secretory cell types have been recognized in the PD in various staining methods including immunocytochemical study [2, 5, 6, 11]. In this study, cells reacting with somatotropin-, prolactin-, ACTH- and LH-antisera are frequently detected in the PD. However, cells immunoreacting to these antisera are undetectable in the PT.

Electron microscopically, two types of secretory cells can be distinguished. Type 1 cells are found in the rostral portion, although the number is not many. They are characterized by the presence of numerous granulated vesicles (150–200 nm in diameter) with variable electron densities (Fig. 4). The Golgi apparatus and rough endoplasmic reticulum are generally well developed. Type 2 cells are found in the caudal portion, and they contain electron-dense granules, mostly with diameters ranging 200 to 360 nm (Fig. 5). They present well developed Golgi apparatus, numerous and dilated cisternae of the rough endoplasmic reticulum. Ultrastructurally, presence of two types of secretory cells in the turtle PT has been reported previously [8]. Judging from the location and the difference in size of granules contained in respective cells and the present immunocytochemical findings, type 1 cells may be characterized as TSH-cells, whereas type 2 cells may be considered as FSH-cells.

The vascularization of the turtle hypophysis has been investigated previously, and portal vessels run through the rostral and caudal PT [12]. Although some cells of type 1 and type 2 are closely contacted with the capillaries of the portal system, extrusion of the secretory granules into the capillaries were not demonstrated. At present, it is not known to which extent these immunoreactive TSH- and FSH-cells in the PT are physiologically involved in the thyrotropic and gonadotropic functions of the pituitary. Presence of immunoreactive TSH- and FSH-cells has been reported in the PT of a variety of mammals [13].

FIGS. 1–3. Mid-sagittal sections through infundibulum and hypophysis. 1. Section stained with Azan stain. Pars tuberalis can be divisible into rostral (rPT) and caudal portion (cPT). Dotted line represents the boundary between rPT and cPT. CE, cephalic lobe of the pars distalis; ME, median eminence. Arrows indicate blood capilaries. ×115. 2. Section immunostained with anti-TSH. Cells reacting to the antiserum are confined to the rPT. ×220. 3. Section immunostained with anti-FSH. Cells reacting to the antiserum are common in the cPT. CA, caudal lobe of the pars distalis. ×220.

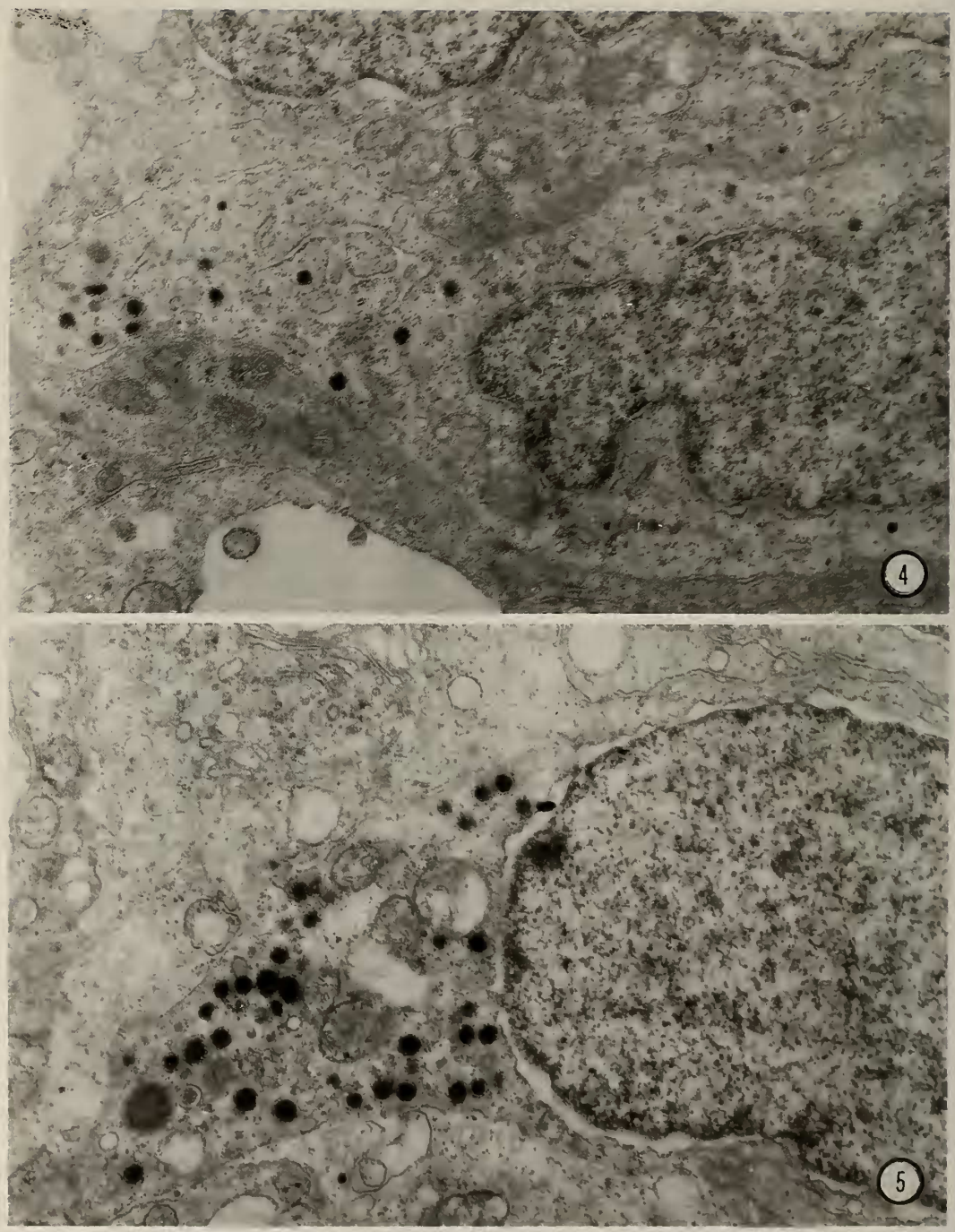

Fig. 4. Electron micrograph of the rostral portion of the PT. Note type 1 cell containing a few granulated vesicles. ×18,200.

Fig. 5. Electron micrograph of outermost layer of the caudal portion of the PT. Note type 2 cell with many dark granules. ×18,200.

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